Staining is a technique used to increase the magnification in the microscopic image. Special stains are used to stain specific structures in the skin. Most common stains used in Dermatopathology are Haemotoxylin and Eosin. Here are a few important stains used in Dermatopathology which are named after a person.

1. Feulgen stain:
Stains DNA, named after Robert Fuelgen (1884-1955), who was a German physician and chemist. He was a pioneer who developed staining method for DNA in 1914.

2. Fite-Faraco stain:
This stain is mainly used to identify partially acid fast organisms such as Mycobacterium Leprae, Atypical Mycobacteria and Nocardia. Stain was named after George Liddle Fite, an American pathologist (1933-1993).

3. Giemsa stain:
This stain is mainly used in cytogenetics. Giemsa’s solution is a mixture of Methylene blue, eosin and Azure B. It is specific for the Phosphate groups of DNA and it is used in giemsa banding also called as G banding which is mainly used to identify chromosomal aberrations.

It is mainly used in the demonstration of parasites such as Plasmodium, Trypanosoma, Chlamydia, and Trichomonas vaginalis.

It is used to demonstrate acantholytic cells, inclusion bodies.

Giemsa stain is a classic blood film stain for bone marrow specimens and peripheral blood smears.

This stain was developed by a German chemist and bacteriologist Gustav Giemsa (1867-1948).

4. Gomori’s methenamine silver stain:
This stain is used for histologic visualisation of fungi, basement membrane and some opportunistic organisms such as Pneumocystis carinii.

This stain also visualises Actinomyces, Nocardia asteroids and few encapsulated bacteria.

The mucopolysaccharide component of the fungus cell wall are oxidized to release aldehyde groups, they react with the silver nitrate, reducing it to metallic silver, making them visible.

REAGENTS:
2% chromic acid, 1% Sodium metabisulfite, 5% Borax ,3% Methenamine, .5% Silver nitrate and 0.5% Gold chloride.

INTERPRETATION:
Fungi- Black
Pneumocystis carinii- black
Mucin- Gray

It was named after George Gomori (1904-1957), who was a Hungarian-American physician who later became famous as histochemist.

5. Gram’s staining:
It is a method of staining which differentiates bacteria into Gram-positive and Gram-negative. This was named after Hans Christian Gram (1853-1958), who was a Danish bacteriologist.

Gram-positive bacteria have a thick cell wall made of peptidoglycan, retain crystal violet and appear purple; whereas gram-negative bacteria have a thinner layer and does not retain purple colour and are counter stained with Saffranin.

METHOD:
1. Applying a primary stain (Crystal Violet) to a heat fixed smear.
2. The addition of Iodide which acts as a mordant, binds to crystal violet and traps it inside the cell.
3. Rapid decolorization with ethanol or acetone.
4. Counter – staining with Safranin or Carbol Fuschin.

INTERPRETATION:
Gram-positive bacteria- violet
Gram-negative bacteria- pink
6. Masson’s trichrome stain:
Masson’s trichrome is a three colour staining technique used in dermatopathology.

The trichrome stain is prepared by immersing fixed sample into Weigert’s iron hematoxylin.

Weigert’s hematoxylin is a sequence of three solutions:
1. Ferric chloride in diluted hydrochloric acid.
2. Hematoxylin in 95% ethanol
3. Potassium ferricyanide solution alkalinised by sodium borate.

INTERPRETATION:
Collagen—blue or green
Keratin and muscle fibers – red
Cell nuclei—black

It was named after French born Canadian pathologist Claude L. Pierre Masson (1880-1957).

7. Verhoeff-Van Gieson stain:
It was developed by Verhoeff (1874-1968) who was an American ophthalmic surgeon and pathologist. Thompson Van Gieson (1866-1913) was an American neuropsychiatrist and pathologist in 1908.

It is mainly used to demonstrate normal or pathologic elastic fibers.

REAGENTS:
Haemotoxylin
Lugol’s iodine
Iron chloride
Van Gieson’s stain
Picric acid
Acid Fuschin
Sodium thiosulfate

INTERPRETATION:
Elastic fibers—black
Collagen fibers—red
Cytoplasm—yellow.

8. Von-Kossa stain:
This stain is used to visualize calcium deposits in tissue sections.

It was developed by Von Kossa in 1901.

It is based on the principle of precipitation reaction where in the addition of silver nitrate solution leads to deposition of silver by replacing calcium reduced by the strong light.

INTERPRETATION:
Calcium—black
Nuclei—red
Cytoplasm—light pink
It is not specific for calcium.

9. Warthin-Starry stain:
It is a silver – nitrate based staining method

It was first introduced in 1920 by two American pathologists, Aldred Scott Warthin (1866-1931) and Allen Chronister Stary (1890-1973) for the detection of spirochetes.

INTERPRETATION:
Helicobacter pylori—black
Legionella pneumophilia—black
Spirochetes—black
Bartonella henselae—black.

10. Ziehl–Neelsen stain:
It is also called as acid-fast stain, which is used to identify acid-fast bacteria. It was first described by two German doctors, Dr Franz Ziehl (1857-1926) and Friedrich Carl Adolf Neelsen (1854-1898).

It is a differential stain used to identify acid – fast organisms, mainly mycobacteria.

COMPONENTS:
1. Primary stain: 0.3% Carbol Fuschin
2. Decolorising agent – Sulphuric acid
3. Counter stain – 0.3% methylene blue or malachite green.

PROCEDURE:
1. Make a thin smear and heat fix by passing the slide 3-4 times over the flame, place the slide on staining rack and pour carbol fuschin over smear and heat gently by placing a flame until fumes appear.
2. Rinse smears with water until no color appears in the effluent.
3. Pour 20% sulphuric acid
4. Wash well with clean water
5. Cover the smear with methylene blue or malachite green stain for 1-2 min
6. Wash off the stain with clean water, allow it to air dry and observe under oil immersion field.

MODIFICATIONS:
1. Use of alcohol as secondary decoloriser- To specify M. Leprae which is both alcohol and acid fast.
2. Use of acid alcohol -3% Hydrochloric acid in 95% alcohol can be used in differentiating M. Tuberculosis.
3. Modifications in percentage of sulfuric acid.
   a. <5% - M. Leprae
   b. 1%-Actinomyces
   c. 0.5%- Nocardia
   d. 0.25-0.5% - spores and oocysts of cryptosporidium and Isospora.

INTERPRETATION:
Acid-fast bacilli appears red, straight or slightly curved rods, occurring singly or in small groups, may appear beaded.

REFERENCES: